

Appl. No. 09/497,943
Response dated May 1, 2006
Reply to Office Action of April 13, 2006

LISTING OF CLAIMS

This listing of claims will replace all prior versions of claims in the application.

1.-28. (Canceled)

29. (Currently amended) A method of labeling an oligonucleotide, comprising the steps of:

- (a) hybridizing a first oligonucleotide to a second oligonucleotide, wherein the first oligonucleotide consists of, from 3' to 5': a Substrate Hybridization Domain adjoining a Signal Template Domain, wherein:
 - i) the Substrate Hybridization Domain consists of a sequence of about 5 to about 20 nucleotides and cannot be extended by a 5' → 3' DNA polymerase; and
 - ii) the Signal Template Domain consists of a sequence of about 5 to about 100 nucleotides;and the second oligonucleotide comprises; from 3' to 5': a Template Hybridization Domain adjoining a Target Binding Domain, wherein:
 - i) the Template Hybridization Domain consists of a sequence of about 5 to about 20 nucleotides which is not detectably labeled, has 5 or more bases complementary to the Substrate Hybridization Domain of the first oligonucleotide, and is hybridizable to the Substrate Hybridization Domain of the first oligonucleotide; and
 - ii) the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain and to that of the first oligonucleotide; and
- (b) extending the second oligonucleotide with a DNA polymerase in the presence of [[a]] labeled nucleotides to create an oligonucleotide Probe having from 5' to 3' an unlabeled Target Binding Domain adjoining a Template Hybridization Domain adjoining a labeled Signal Domain.

Appl. No. 09/497,943
Response dated May 1, 2006
Reply to Office Action of April 13, 2006

30. (Currently amended) The method of claim 29, wherein the ~~nucleotides which comprise the first or second oligonucleotide~~ are consists of deoxyribonucleotides.

31. (Currently amended) The method of claim 29, wherein the ~~nucleotides which comprise the first or second oligonucleotide~~ are consists of ribonucleotides.

32. (Previously presented) The method of claim 29, wherein the second oligonucleotide consists of about 15 to about 150 nucleotides.

33-36. (Canceled)

37. (Currently amended) The method of claim ~~35~~ 29, wherein the 3' nucleotide of the Substrate Hybridization Domain ~~comprises~~ is a 3' -terminal modified nucleotide.

38. (Original) The method of claim 37, wherein the modification is selected from the group consisting of: a 3' -amino-modifier, a 2', 3' -dideoxynucleotide, a 3' -phosphate, and a modified 3' -phosphate group.

39. (Currently amended) The method of claim 29, wherein the 3' nucleotide of the Substrate Hybridization Domain ~~comprises at least one nucleotide which comprises~~ is a modified cytidine, ~~which nucleotide is~~ selected from the group consisting of: C5-methyl-dC and C5-propynyl-dC.

40. (Currently amended) The method of claim 29, wherein the Signal Template Domain consists ~~essentially~~ of about 10 to about 50 nucleotides.

41. (Previously presented) The method of claim 29, wherein the Signal Domain is at least 50% homopolymeric.

42. (Canceled)

Appl. No. 09/497,943
Response dated May 1, 2006
Reply to Office Action of April 13, 2006

43. (Previously presented) The method of claim 41, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

44. (Original) The method of claim 29, wherein the extending step is carried out by a DNA polymerase selected from the group consisting of: E. coli DNA polymerase I holoenzyme, Klenow fragment of E. coli DNA polymerase I, T4 DNA polymerase, T7 DNA polymerase, and a DNA polymerase encoded by a thermophilic bacterium.

45. (Currently amended) The method of claim 29, wherein one or more of the nucleotides of the Template Hybridization Domain or the Substrate Hybridization Domain ~~comprises at least one~~ is a modified nucleotide, which modified nucleotide increases the hybridization affinity of said Template Hybridization Domain to said Substrate Hybridization Domain.

46. (Original) The method of claim 45, wherein at least one modified nucleotide is found in the Template Hybridization Domain.

47. (Original) The method of claim 46, wherein at least one modified nucleotide is selected from the group consisting of: C5-methyl-dC, C5-propynyl-dC, C5-propynyl-dU, and 2,6 diaminopurine.

48. (Currently amended) The method of claim 29, wherein at least one nucleotide ~~comprises~~ is labeled with a label selected from the group consisting of: ^{32}P , ^{33}P , ^{35}S , fluorescein, digoxigenin, biotin, Cy5, Cy3, and rhodamine.

49.-54. (Canceled)

55. (Previously presented) The method of claim 29, wherein the oligonucleotide Probe has a specific activity of 7×10^7 CPM per picomole.

56. (Previously presented) The method of claim 29, wherein the oligonucleotide Probe has a specific activity of 9×10^7 CPM per picomole.

Appl. No. 09/497,943
Response dated May 1, 2006
Reply to Office Action of April 13, 2006

57. (Canceled)

58. (Previously presented) The method of claim 29, wherein the Signal Domain is at least 70% homopolymeric.

59. (Previously presented) The method of claim 29, wherein the Signal Domain is at least 90% homopolymeric.

60. (Previously presented) The method of claim 29, wherein the Signal Domain is 100% homopolymeric.

61. (Currently amended) The method of claim 29, wherein the Substrate Hybridization Domain consists essentially of a sequence of from about 5 to about 10 nucleotides and wherein the Template Hybridization Domain consists essentially of a sequence of from about 5 to about 10 nucleotides.

62. (Canceled)

63. (Currently amended) The method of claim 62 29, wherein the second oligonucleotide consists essentially of a sequence of about 15 to about 150 nucleotides.

64. (Currently amended) A method of labeling an oligonucleotide, comprising the steps of:

- (a) hybridizing a first oligonucleotide to a second oligonucleotide, wherein the first oligonucleotide consists of, from 3' to 5', a 3' nucleotide extension overhang ~~having no complementarity to the Template Hybridization Domain of the second oligonucleotide~~ adjoining a Substrate Hybridization Domain adjoining a Signal Template Domain, wherein:
 - i) the Substrate Hybridization Domain consists of a sequence of about 5 to about 10 nucleotides and cannot be extended by a 5' → 3' DNA polymerase; and

Appl. No. 09/497,943
Response dated May 1, 2006
Reply to Office Action of April 13, 2006

- ii) the Signal Template Domain consists of a sequence of about 5 to about 100 nucleotides;
and the second oligonucleotide comprises, from 3' to 5': a Template Hybridization Domain adjoining a Target Binding Domain, wherein:
 - i) the Template Hybridization Domain consists of a sequence of about 5 to about 10 nucleotides, is not detectably labeled, and has at least 5 bases complementary to the Substrate Hybridization Domain of the first oligonucleotide;
 - ii) the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain; and
- (b) extending the second oligonucleotide with a DNA polymerase in the presence of labeled nucleotides to form an oligonucleotide probe having from 5' to 3' an unlabeled Target Binding Domain adjoining a Template Hybridization Domain adjoining a labeled Signal Domain,
wherein the overhang on the first oligonucleotide blocks extension of the first oligonucleotide by the DNA polymerase.